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EDITORIAL COMMENT

Molecular Imaging

Antidote to Cardiac Stem Cell Controversy*

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Ischemic heart disease is the leading cause of morbidity and mortality in the U.S. and industrialized countries (1). In acute myocardial infarction, tissue loss leads to hemodynamic stress followed by compensatory left ventricular hypertrophy and dilation (2). In recent years, several studies have shown that transplantation of skeletal myoblasts (3), bone marrow cells (4,5), mesenchymal stem cells (6), endothelial progenitor cells (7), cardiac resident stem cells (8–10), and embryonic stem cell derivatives (11–13) can accelerate the myocardial regenerative process. The mechanism(s) may be related to stem cells secreting multiple angiogenic factors, providing a mechanical scaffold, and/or recruiting other beneficial cells to the ischemic territory (14). However, results from more recent large randomized clinical trials related to circulating progenitor cells (the TOPCARE-CHD [Transplantation of Progenitor Cells and Recovery of LV Function in Patients with Chronic Ischemic Heart Disease] trial) (15), bone marrow cells (the ASTAMI [Autologous Stem-Cell Transplantation in Acute Myocardial Infarction] trial) (16), and skeletal myoblasts (the MAGIC [Myoblast Autologous Grafting in Ischemic Cardiomyopathy] trial) (17) have yielded no significant results. The cause for this disappointment could be due to suboptimal delivery techniques, insufficient engraftment, acute donor cell death, and/or other unknown causes.

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Thus, despite the exciting promise of stem cell therapy, many gaps still exist in the understanding of stem cell biology. This sentiment is reflected by a National Heart, Lung, and Blood Institute workshop on stem cell transplantation, which stated: "It is of critical importance to adopt standard assays. . .to determine whether grafted cells function in a predictable way. . .and to have *molecular markers* that would determine the nature of the engrafted cells and their progeny" (18). Therefore, the development of imaging strategies that can monitor stem cell survival, proliferation, and migration will represent a significant advance in the stem cell field.

Different Imaging Modalities

To date, the majority of studies on stem cell fate have relied on ex vivo analysis such as histologic staining for green fluorescent protein or β-galactosidase. To understand cell fate in vivo, noninvasive techniques must be developed. The *first* approach is to directly label cells with radioactive tracers (e.g., ^{111}Indium or ^{18}F-FDG) and monitor cell trafficking by a gamma camera or single-photon emission computed tomography (SPECT) or positron emission tomography (PET) imaging. The advantages here are the high detection sensitivity of nuclear imaging techniques and possible immediate translation to clinical practice, as most of the radiotracers are already in clinical use. For example, Hofmann et al. (19) isolated bone marrow cells from patients with acute myocardial infarction and radiolabeled them with ^{18}F-FDG. After intracoronary delivery of ^{18}F-FDG–labeled CD34^{+} enriched cells, they found that 14% to 39% of the total activity was detected in the infarcted myocardium after 1 h. However, the short half-lives of ^{18}F (110 min) and most other radioisotopes limit these type of studies to less than 1 to 3 days. The *second* approach is to label cells with iron particles and track cell fate by magnetic resonance imaging (MRI) (20). The main advantage here is MRI's capacity for high anatomic resolution of the sites where cells were injected. However, MRI is unable to distinguish viable from nonviable cells, as iron particles may be retained by living cells, dead cells, or scavenger cells (21–23). Thus, the robust cell signals that persist out to 8 weeks seen in previous studies most likely do not represent viable cells (6). The *third* approach is based on the transfer of various reporter gene constructs into stem cells via a viral or nonviral vector (24). As described in Figure 1, this simple but elegant approach can truly characterize the survival, proliferation, and death of transplanted cells. The same approach was employed by Terrovitis et al. (25) in this issue of the *Journal* to image the fate of transplanted rat cardiac-derived stem cells (rCDCs), also known as resident cardiac stem cells, using a combined SPECT and PET imaging approach.

PET and SPECT Imaging of rCDCs

Terrovitis et al. (25) examined the use of a sodium iodide symporter (NIS) reporter gene to track the fate of transplanted rCDCs. The NIS is normally expressed in the thyroid, stomach, choroid plexus, and salivary gland, but not in the heart. The reporter probes used were technetium 99m and iodine 124 for SPECT and PET imaging, respectively. In addition, thallium 201 (or ammonia 13) radiotracers were used as a road map for myocardial delineation. Their results showed that lentiviral transduction of rCDCs with the NIS reporter gene

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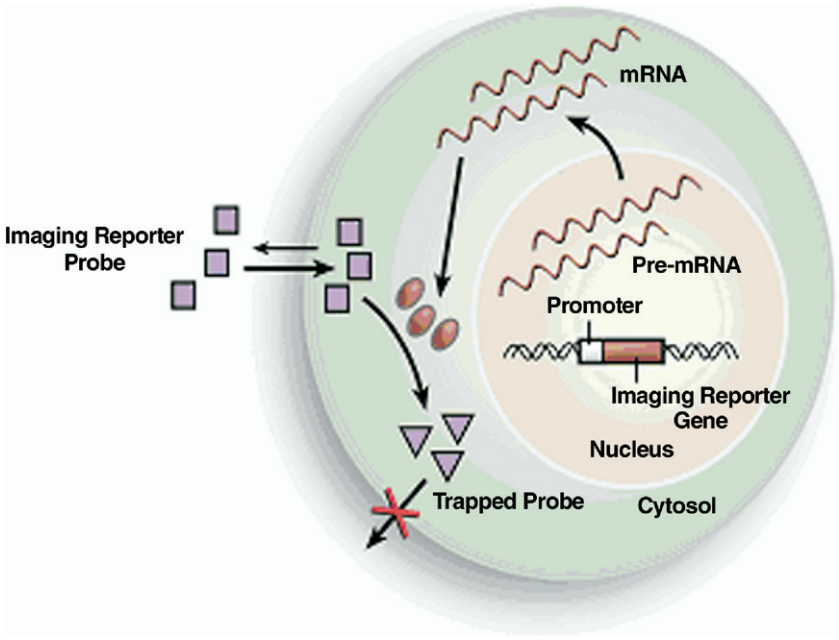

Figure 1 **Conceptual Basis of Reporter Gene and Reporter Probe Imaging for Tracking Stem Cell Fate In Vivo**

In this study, a self-inactivating lentivirus was used to stably transduce rat cardiac-derived stem cells. The cytomegalovirus promoter used to drive the sodium iodide symporter (NIS) reporter gene is constitutively expressed. However, one can also adopt other promoter systems such as inducible (e.g., Tet-on) or tissue-specific (e.g., Nkx2.5) promoters. The reporter gene/reporter probe interactions can be NIS/technetium 99m (for single-photon emission computed tomography imaging), as shown here, or firefly luciferase/D-luciferin (bioluminescence), green fluorescence protein (fluorescence), HSVtk/^{18}F-FHBG (positron emission tomography), and transferrin receptor/iron oxide particles (magnetic resonance imaging), as in other previously published studies. After injection of genetically modified stem cells into the heart, 3 scenarios can happen. If the cells survive and are functionally active, transcription and translation lead to reporter proteins, which interact with the radioactive reporter probes (injected into the animal before scan) to generate detectable imaging signals. If the cells proliferate, the reporter gene will be passed on to daughter cells, and the corresponding imaging signals will increase in intensity. However, if cells are apoptotic or dead, there will be no signals from those cells. mRNA = messenger ribonucleic acid.

did not adversely affect cell viability, proliferation, cardiogenic potential, and angiogenic capacity. After left anterior descending coronary artery ligation of the rat heart, rCDCs ($1 \times 10^6 - 4 \times 10^6$) were injected intramyocardially. The rCDCs were detected by SPECT imaging on days 1, 3, and 6 post-injection but not on day 10 (imaging contrast ratios were 452 ± 29%, 196 ± 71%, 131 ± 66%, and 1.1 ± 14%, respectively). Interestingly, the imaging contrast ratio obtained by PET was less optimal, suggesting a potential limitation of iodine 124 as a reporter probe for NIS.

Importantly, the imaging results indicate that NIS can be used for longitudinal stem cell tracking. They also suggest that the majority of transplanted cells will die within the first 2 weeks, as has been shown previously by other investigators using serial terminal deoxynucleotidyl transferase-mediated dUTP-biotin nick end labeling staining (26), TaqMan polymerase chain reaction of male-specific *Sry* gene (27), and reporter gene imaging (28). Using real-time polymerase chain reaction, the authors also found that 13.2 ± 3.6% of the injected cells were retained on day 1 and 2.8 ± 1.8% at day 8 after injection. This biological phenomenon begs the questions of why a cell type (i.e., rCDCs), which can proliferate so robustly in vitro, tends to die off so acutely after transplantation, as well as whether significant "myocardial regeneration" leading to functional improvement can indeed be achieved using resident cardiac stem cells (or any stem cell type) in future clinical trials. Given that the degree of cell engraftment can vary considerably within the animal cohorts, another area of interest would have been to correlate the survival of rCDCs with the functional changes in individual animals. However, this question was not addressed in this study.

Overall, Terrovitis et al. (25) should be congratulated for performing a meticulous study on the role of NIS reporter gene imaging for tracking stem cell therapy. The NIS provides another alternative reporter gene to the more commonly used herpes simplex virus thymidine kinase (HSVtk). Because NIS is an endogenous mammalian gene, it is expected to be less immunogenic compared with HSVtk (although a newer generation of human-derived nonimmunogenic mitochondrial thymidine kinase type 2 reporter gene has now been developed [29]). However, more detailed studies of the potential adverse effects of NIS on cellular characteristics (beyond the cell proliferation and viability data provided here) will need to be conducted in the future, perhaps similar to what have been done for HSVtk reporter gene using genomics (30) and proteomics (31) analysis. Finally, as the authors pointed out, the feasibility of imaging NIS in a large animal model needs to be demon-

strated before definitive conclusions about clinical translatability can be drawn.

Imaging to Address Biological Questions of Stem Cell Therapy

Besides this elegant report, it is worth pointing out that several recent studies have used the reporter gene imaging approach (Fig. 1) to elucidate stem cell fate and function in vivo. Cao et al. (32) injected mouse embryonic stem cells stably transfected with a trifusion reporter gene (firefly luciferase, red fluorescent protein, and HSVtk) in the rodent heart to demonstrate multimodality imaging of cell survival, cell proliferation, and ablation of cell misbehavior. Similarly, Gyongyosi et al. (33) injected porcine mesenchymal stem cells stably transfected with a variant of the trifusion reporter gene into the pig heart and performed clinical PET imaging to document cellular persistence at 10 days post-delivery. Rodriguez-Porcel et al. (34) showed the feasibility of quantifying regional myocardial transgene expression in a pig model with clinical PET/computed tomographic imaging by using endomyocardial catheter delivery of adenovirus carrying the HSVtk reporter gene. Using a transgenic mouse model that stably expresses firefly luciferase and green fluorescent protein, Sheikh et al. (35) provided insights into the "spatiotemporal kinetics" of how bone marrow cells can home in on ischemic hearts after intravenous delivery. In a follow-up study, van der Bogt et al. (36) performed head-to-head comparisons of the post-intramyocardial transplantation survival and efficacy of bone marrow cells, skeletal myoblasts, and mesenchymal stem cells derived from these colored transgenic mice. Finally, Hung et al. (37) showed that mouse embryonic stem cells delivered into the normal (remote) zone have better cell viability compared with that seen with injections in the intrainfarction and peri-infarction zones.

Conclusions

Despite the potential promise of cardiac stem cell therapy, many fundamental questions have yet to be answered. These include the following issues: 1) What are the molecular and cellular mechanisms of myocardial improvement? 2) What are the optimal cell type, delivery technique, and cell dosage for therapy? 3) How well do transplanted cells survive, integrate, and proliferate in the target organ? 4) What is the long-term fate of transplanted cells—that is, do they engraft or transdifferentiate (or both)? 5) Can these issues be examined via noninvasive imaging rather than invasive biopsy or post-mortem histology? From the studies highlighted in the previous text, it is becoming clear that molecular imaging will play an ever growing role in answering these questions. It is also quite heartening to see how a field that was originally developed by our oncology colleagues to track cancer gene/cell therapy (38,39) has now gradually gained acceptance by the cardiovascular community over the past 5 years. The challenge in the next 5 years will be to implement clinical molecular imaging to help fulfill the promise of cardiac stem cell therapy, as well as to resolve the disappointments and controversies surrounding the field.

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